Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis

Rao, Deepak A.; Gurish, Michael F.; Marshall, Jennifer L.; Slowikowski, Kamil; Fonseka, Chamith Y.; Liu, Yanyan; Donlin, Laura T.; Henderson, Lauren A.; Wei, Kevin; Mizoguchi, Fumitaka; Teslovich, Nikola C.; Weinblatt, Michael E.; Massarotti, Elena M.; Coblyn, Jonathan S.; Helfgott, Simon M.; Lee, Yvonne C.; Todd, Derrick J.; Bykerk, Vivian P.; Goodman, Susan M.; Pernis, Alessandra B.

DOI:
10.1038/nature20810

Citation for published version (Harvard):
Rao, DA, Gurish, MF, Marshall, JL, Slowikowski, K, Fonseka, CY, Liu, Y, Donlin, LT, Henderson, LA, Wei, K, Mizoguchi, F, Teslovich, NC, Weinblatt, ME, Massarotti, EM, Coblyn, JS, Helfgott, SM, Lee, YC, Todd, DJ, Bykerk, VP, Goodman, SM, Pernis, AB, Ivashkiv, LB, Karlson, EW, Nigrovic, PA, Filer, A, Buckley, CD, Lederer, JA, Raychaudhuri, S & Brenner, MB 2017, 'Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis', *Nature*, vol. 542, no. 7639, pp. 110-114. https://doi.org/10.1038/nature20810

Pathologically expanded 'peripheral' B cell-helper T cells in rheumatoid arthritis

Deepak A. Rao[1], Michael F. Gurish[1], Jennifer L. Marshall[2], Kamil Slowikowski[1,3,4,5,6], Chamith Fonseka[1,3,4,6,7], Yanyan Liu[1], Laura T. Donlin[8,9], Lauren A. Henderson[10], Kevin Wei,[1] Fumitaka Mizoguchi[1], Nikola C. Teslovich[1,3,4], Michael E. Weinblatt[1], Elena M. Massarotti[1], Jonathan S. Coblyn[1], Simon M. Helfgott[1], Yvonne C. Lee[1], Derrick J. Todd[1], Vivian P. Bykerk[11,12], Susan M. Goodman[11,12], Alessandra B. Pernis[9,12,13], Lionel B. Ivaskiv[8,9], Elizabeth W. Karlson[1], Peter A. Nigrovic[1,10], Andrew Filer[2], Christopher D. Buckley[2], James A. Lederer[14], Soumya Raychaudhuri[1,3,4,5,15,16], and Michael B. Brenner[1]

1) Division of Rheumatology, Immunology, and Allergy, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115 USA

2) Rheumatology Research Group, Institute of Inflammation and Ageing, University of Birmingham, Queen Elizabeth Hospital, Birmingham, B15 2WB, UK

3) Division of Genetics, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115 USA

4) Program in Medical and Population Genetics, Broad Institute of Massachusetts Technical Institute and Harvard University, Cambridge, MA 02138, USA

5) Partners Center for Personalized Genetic Medicine, Boston, MA 02115 USA

6) Bioinformatics and Integrative Genomics, Harvard University, Cambridge, MA 02138, USA

7) Biological and Biomedical Sciences, Harvard University, Cambridge, MA 02138, USA

8) Arthritis and Tissue Degeneration Program, Hospital for Special Surgery, New York, New York 10021, USA

9) David Z. Rosensweig Genomics Research Center, Hospital for Special Surgery, New York, New York 10021, USA

10) Division of Immunology, Boston Children's Hospital, Boston, MA 02115 USA

11) Division of Rheumatology, Hospital for Special Surgery, 535 E 70th Street, New York, NY 10021

12) Department of Medicine, Weill Cornell Medical College, Cornell University, New York, NY 10021, USA

13) Autoimmunity and Inflammation Program, Hospital for Special Surgery, New York, NY 10021, USA

14) Department of Surgery, Brigham and Women's Hospital, Boston, MA 02115 USA

15) Reumatology Unit, Karolinska Institutet and Karolinska University Hospital Solna, Stockholm, Sweden

16) Institute of Inflammation and Repair, University of Manchester, Manchester, UK

Keywords: follicular helper T cells, rheumatoid arthritis, PD-1, plasma cells, CCR2, mass cytometry

SUMMARY

CD4$^+$ T cells are central mediators of autoimmune pathology; however, the definition of their key effector functions in specific autoimmune diseases remains limited. Pathogenic CD4+ T cells within affected tissues may be identified by expression of markers of recent activation[1]. We applied this approach to joint tissue in rheumatoid arthritis (RA), a chronic immune-mediated arthritis that affects up to 1% of the population[2]. Utilizing mass cytometry to detect activated T cells in RA synovial tissue revealed a strikingly expanded population of PD-1^{hi} CXCR5$^-$ CD4$^+$ T cells. These cells are not exhausted, Rather, multidimensional cytometry, transcriptomics, and functional assays define a population of PD-1^{hi} CXCR5$^-$ 'peripheral helper' T (Tph) cells that express factors enabling B cell help, including IL-21, CXCL13, ICOS, and MAF. Like PD-1^{hi} CXCR5$^+$ T 'follicular helper' (Tfh) cells, Tph cells induce plasma cell differentiation *in vitro* via IL-21 and SLAMF5-interactions[3,4]. However, global transcriptomics robustly separate Tph cells from Tfh cells, with altered expression of Bcl6 and Blimp-1 and unique expression of chemokine receptors that direct migration to inflamed sites, such as CCR2, CX3CR1, and CCR5, in Tph cells. Tph cells appear uniquely poised to promote B cell responses and antibody production within pathologically inflamed non-lymphoid tissues.

We analyzed $CD4^+$ T cells in 3 seropositive (defined as rheumatoid factor+ or anti-citrullinated peptide antibody+) RA synovial tissue samples with dense leukocyte infiltrates using a mass cytometry panel designed to interrogate both stromal and leukocyte populations (**Extended Data Table 1**). Two-dimensional visualization of the multidimensional cytometry data using the viSNE algorithm[5] revealed a heterogeneous $CD4^+$ T cell population with distinct expression patterns of 5 commonly used activation markers (PD-1, MHC II, ICOS, CD69, CD38) (**Fig. 1a**). Strikingly, a large population of cells with high PD-1 expression clustered together in each of the 3 samples (**Fig. 1a**, **Extended Data Fig. 1a**). Biaxial gating of data from 6 seropositive RA synovial tissue samples confirmed high expression of PD-1 on ~25% of synovial $CD4^+$ T cells, the majority of which co-expressed MHC II and/or ICOS (**Fig. 1b**, **Extended Data Fig. 1b, Extended Data Table 2**).

In a complementary approach, 11-dimensional flow cytometric analysis of memory $CD4^+$ T cells from paired synovial fluid and blood samples from 3 seropositive RA patients also revealed a large population of synovial PD-1^{hi} $CD4^+$ T cells, a subset of which co-expressed MHC II and/or ICOS (**Fig. 1c, Extended Data Table 3**). Biaxial gating confirmed high PD-1 expression on ~30% of synovial fluid $CD4^+$ T cells, mirroring results from synovial tissue (**Fig. 1d,e, Extended Data Fig. 1c**). The frequency of PD-1^{hi} $CD4^+$ T cell populations in seropositive RA synovial fluid (n=9) was over 5-fold higher than in synovial fluid from 19 patients with seronegative inflammatory arthritides (seronegative RA n=2, spondyloarthropathy n=8, juvenile idiopathic arthritis n=9, $p<0.0001$, Mann-Whitney) (**Fig. 1d,e**).

Because seropositive RA is characterized by autoantibody production and frequent synovial T cell-B cell aggregates[6,7], we considered whether synovial PD-1^{hi} cells might be Tfh cells. Tfh cells, often identified as CXCR5^{+} PD-1^{+}, are uniquely adapted to promote B cell recruitment and differentiation in lymph node follicles via production of IL-21, IL-4, CD40L, and CXCL13, the ligand for CXCR5[4]. However, seropositive RA synovial tissue samples contained few PD-1^{hi} CXCR5^{+} Tfh cells (**Fig. 1f,g**), which clustered separately from PD-1^{hi} CXCR5^{-} cells in viSNE analyses (**Fig. 1a**, right panel). In contrast, ~85% of PD-1^{hi} cells in synovial tissue lacked CXCR5, as did almost all PD-1^{hi} cells in synovial fluid (**Fig. 1f,g)**. Measurement of CXCR5 transcript levels in sorted PD-1^{hi} CXCR5^{-} and PD-1^{hi} CXCR5^{+} cells from synovial tissue, synovial fluid, and blood confirmed that PD-1^{hi} CXCR5^{-} cells from all 3 sources contained little, if any, CXCR5 mRNA (**Extended Data Fig. 1d,e**). Thus, seropositive RA synovium contains abundant PD-1^{hi} CD4^{+} T cells that are not Tfh cells.

Intriguingly, PD-1^{hi} CXCR5^{-} CD4^{+} T cells with a similar multidimensional phenotype also appeared in the circulation, albeit at much lower frequencies (**Fig. 1c, Extended Data Fig. 2a**). Quantification of circulating PD-1^{hi} CXCR5^{-} memory CD4^{+} T cells in patients with established seropositive RA (n=42), seronegative RA (n=16), spondyloarthropathies (n=11), and non-inflammatory controls (n=35) demonstrated a significantly increased frequency of PD-1^{hi} CXCR5^{-} cells specifically in seropositive RA patients (**Fig. 1h, Extended Data Fig. 2b**, patient characteristics in **Extended Data Table 2**). PD-1^{hi} MHC II^{+} CXCR5^{-} and PD-1^{hi} ICOS^{+} CXCR5^{-} cells were also increased in blood of seropositive RA patients (**Extended Data Fig. 2c**). In contrast, the frequencies of PD-1^{hi} CXCR5^{+} cells and cells with intermediate PD-1 expression were not increased (**Extended Data Fig. 2d,e**).

PD-1^{hi} CXCR5^{-} cell frequencies were more robustly increased in seropositive RA patients with moderate or high disease activity (clinical disease activity index (CDAI)>10), compared to patients with low disease activity (CDAI≤10) (**Fig. 1i**). The frequency of PD-1^{hi} CXCR5^{-} cells did not vary with other clinical parameters such as age, sex, disease duration, use of methotrexate or biologic therapies, or serum anti-CCP antibody titer (**Extended Data Fig. 2f-h**). In an independent cohort of 23 seropositive RA patients assayed before and after starting a new RA medication, there was a significant correlation between reduction in disease activity and reduction in the frequency of PD-1^{hi} CXCR5^{-} T cells (**Extended Data Fig. 2i**). The frequency of PD-1^{hi} CXCR5^{-} cells, PD-1^{hi} MHC II^{+} CXCR5^{-} and PD-1^{hi} ICOS^{+} CXCR5^{-} cells decreased significantly in the 18 patients whose disease activity improved after treatment escalation (**Fig. 1j, Extended Data Fig. 2j**).

Since high PD-1 expression is often considered indicative of an exhausted state[8,9], we assessed the function of synovial PD-1^{hi} CXCR5^{-} cells. Surprisingly, despite lack of CXCR5, PD-1^{hi} CD4^{+} T cells sorted from seropositive RA synovial fluid showed >100-fold increased mRNA expression of IL-21 and >1000-fold increased expression of CXCL13, as well as higher levels of IFN-γ and IL-10, compared to PD-1^{-} T cells, with the highest expression in PD-1^{hi} MHC II^{+} cells (**Fig. 2a,** sorted as in **Extended Data Fig. 1d**). In contrast, IL-2 showed a trend towards lower expression in PD-1^{hi} cells.

Consistent with mRNA expression, PD-1^{hi} CXCR5^{-} cells sorted from RA synovial fluid more frequently produced IL-21 (~30%), but less frequently produced IL-2, compared to PD-1^{-} or PD-1^{int} cells, after stimulation with PMA+ionomycin (**Fig. 2b**). Optimal CXCL13 production was detected after 24 hours of stimulation with anti-CD3/CD28 beads. Strikingly, at this timepoint, ~25% of PD-1^{hi} CXCR5^{-} cells produced CXCL13, but not IL-2, compared to <1% of PD-1^{-} or PD-1^{int} cells. High IL-21 and CXCL13 production by synovial fluid PD-1^{hi} CXCR5^{-} cells indicates that these cells are not globally exhausted, and instead suggested possible B cell-helper function.

In support of possible B cell helper-function, PD-1^{hi} MHC II^{+} cells in seropositive RA synovial fluid also expressed high mRNA levels of the transcription factors MAF and BATF and the signaling adaptor SAP (encoded by SH2D1A). These 3 factors are important for Tfh cell development or function (**Fig. 2a**)[4]. However, Bcl6, a transcription factor characteristically expressed in Tfh cells, was not elevated in synovial fluid PD-1^{hi} cells, while Blimp-1, a transcription factor typically downregulated in Tfh cells, was upregulated[4,10].

Intracellular flow cytometry confirmed that Blimp-1 was significantly elevated in PD-1^{hi} CXCR5^{-} cells, but not PD-1^{hi} CXCR5^{+} cells, from seropositive RA synovial samples (**Fig. 2c**). In contrast, Bcl6 was dramatically elevated in PD-1^{hi} CXCR5^{+} cells, such that the Bcl6/Blimp-1 ratio was uniquely elevated in synovial PD-1^{hi} CXCR5^{+} cells. Expression of MAF, a factor that promotes IL-21 production in human CD4^{+} T cells[11], was elevated in both PD-1^{hi} CXCR5^{-} and PD-1^{hi} CXCR5^{+} cells.

PD-1^{hi} CD4^{+} T cells from peripheral blood showed a transcriptional pattern similar to that in synovial fluid PD-1^{hi} cells, with increased expression of IL-21, CXCL13, IFN-γ, MAF, SAP, and Blimp-1, but not IL-2 or Bcl6, in circulating PD-1^{hi} MHC II^{+} cells compared to PD-1^{-} cells (**Extended Data Fig. 3a,b**). Both PD-1^{hi} CXCR5^{-} and PD-1^{hi} CXCR5^{+} cells expressed increased IL-21 and CXCL13 and decreased IL-2 compared to PD-1^{-} T cells (**Extended Data Fig. 3b**). However, Blimp-1 expression was ~3-fold higher in blood PD-1^{hi} CXCR5^{-} cells compared to PD-1^{hi} CXCR5^{+} cells, while Bcl6 expression was similar. Consistently, after *in vitro* stimulation, blood PD-1^{hi} CXCR5^{-} cells expressed more Blimp-1 and less Bcl6 protein than did PD-1^{hi} CXCR5^{+} populations (**Extended Data Fig. 3c***).* Taken together, these results indicate that both synovial and blood PD-1^{hi}

CXCR5^{-} cells express factors associated with B cell-helper function without an elevated Bcl6/Blimp-1 expression ratio.

To compare PD-1^{hi} CXCR5^{-} and PD-1^{hi} CXCR5^{+} cells more broadly, we analyzed blood PD-1^{hi} cells by mass cytometry (**Extended Data Table 1**). viSNE visualization of blood CD4^{+} T cells clustered PD-1^{hi} CXCR5^{-} and PD-1^{hi} CXCR5^{+} cells in close proximity, indicating a similar multidimensional phenotype (**Fig. 3a**, **Extended Data Fig. 4a**). In contrast, FoxP3^{+} T regulatory cells aggregated in a separate region, indicating that most PD-1^{hi} cells are not T regulatory cells, a finding confirmed by flow cytometry (**Fig. 3a**, **Extended Data Fig. 4b**).

Both PD-1^{hi} CXCR5^{-} cells and PD-1^{hi} CXCR5^{+} cells showed significantly increased expression of 11 proteins, including TIGIT, ICOS, CD38, and CD57, and significantly decreased expression of 5 proteins, including CD25 and CD127 (**Fig. 3b**). Unlike TIGIT, additional inhibitory receptors TIM-3, LAG-3, and CTLA-4 did not appear enriched on PD-1^{hi} CXCR5^{-} cells (**Extended Data Fig. 4c**). Compared to PD-1^{hi} CXCR5^{+} cells, PD-1^{hi} CXCR5^{-} cells showed lower expression of CCR7 and CD27 but higher CD44 and T-bet (**Fig. 3b, c**), suggesting a potentially distinct migratory capacity[12,13].

We next performed an unbiased transcriptomic comparison of blood PD-1^{hi} CXCR5^{-} and PD-1^{hi} CXCR5^{+} cell subpopulations by low-input RNA sequencing[14]. Principal components analysis (PCA) revealed that PD-1^{hi} populations that co-expressed ICOS and/or MHC II were similarly separated from PD-1^{-} cells along the first principal component (PC1), irrespective of CXCR5 expression (**Fig. 3d, gated in Extended Data Fig. 4d**). However, PD-1^{hi} CXCR5^{-} and PD-1^{hi} CXCR5^{+} cell populations were largely distinguished by the second principal component (PC2), indicating considerable differences in the global transcriptomes of PD-1^{hi} CXCR5^{-} cells and PD-1^{hi} CXCR5^{+} cells beyond CXCR5 expression alone.

Sixty-six genes were significantly differentially expressed when comparing all of the PD-1^{hi} populations to the PD-1^{-} populations (log fold change >1.2, FDR<0.01, **Extended Data Table 4**), including MAF, TIGIT, and SLAMF6[15,16]. Analysis of a curated list of Tfh-associated genes[15,17,18] demonstrated similar upregulation of multiple genes in the pooled PD-1^{hi} CXCR5^{+} cell samples and PD-1^{hi} CXCR5^{-} cell samples (**Fig. 3e**), and hierarchical clustering of all 8 cell populations based on this gene list perfectly segregated all PD-1^{hi} populations from PD-1^{-} populations, regardless of CXCR5 expression (p<0.026, **Extended Data Fig. 4e**). These results highlight a shared transcriptional program associated with B cell-helper function in PD-1^{hi} CXCR5^{-} cells and Tfh cells.

However, we also identified 16 genes with significantly different expression between PD-1^{hi} CXCR5^{-} and PD-1^{hi} CXCR5^{+} cells (**Extended Data Table 5**). Notably, PD-1^{hi} CXCR5^{-} cells showed 34-fold increased expression of CCR2, a chemokine receptor that mediates migration to sites of peripheral inflammation[19]. A targeted analysis of chemokine receptor expression on PD-1^{hi} CXCR5^{-} cells demonstrated striking upregulation of a set of ‘inflammatory’ chemokine receptors on these cells, including CCR2, CX3CR1, and CCR5, which was confirmed by flow cytometry (**Fig. 3f,g**)[20]. Notably, ~50% of PD-1^{hi} CXCR5^{-} cells in seropositive RA synovial fluid and synovial tissue expressed CCR2 (**Fig. 3h**). These results indicate that PD-1^{hi} CXCR5^{-} cells can be distinguished from PD-1^{hi} CXCR5^{+} cells not only by the lack of CXCR5 but also by high expression of inflammatory chemokine receptors.

To investigate the interconversion of PD-1^{hi} cells that express distinct chemokine receptors, PD-1^{hi} CXCR5^{-} CCR2^{-}, PD-1^{hi} CXCR5^{-} CCR2^{+}, and PD-1^{hi} CXCR5^{+} CCR2^{-} cell populations sorted from blood were stimulated *in vitro* and re-evaluated at different timepoints (**Extended Data Fig. 5a,b**). At day 2, CXCR5 was transiently induced on both naïve and memory CD4^{+} T cell populations (**Extended Data Fig. 5c**), as previously described[21]. Interestingly, PD-1^{hi} CCR2^{+} cells showed the most limited induction of CXCR5. By day 7, the majority of PD-1^{hi} cells that started out CXCR5^{-} CCR2^{+} cells remained CCR2^{+}, while less than 5% of these cells expressed CXCR5 (**Extended Data Fig. 5d**). Conversely, most PD-1^{hi} cells that started out CXCR5^{+} CCR2^{-} remained CXCR5^{+}, and less than 5% of these cells acquired CCR2 These results demonstrate that even with powerful TCR stimulation, CXCR5 and CCR2 expression remain persistent, distinguishing features on PD-1^{hi} *in vitro*.

We next tested directly if PD-1^{hi} CXCR5^{-} CD4^{+} T cells can provide B cell help *in vitro*. PD-1^{hi} CXCR5^{-} cells sorted from seropositive RA synovial tissue or synovial fluid induced differentiation of co-cultured memory B cells into plasma cells, while CXCR5^{-} cells without high PD-1 expression did not (**Fig. 4a,b**, sorted as in **Extended Data Fig. 1d**). The limited number of CXCR5^{+} T cells in synovial samples precluded comparison with PD-1^{hi} CXCR5^{+} cells. PD-1^{hi} CXCR5^{-} cells from blood also induced memory B cell differentiation into plasma cells, with comparable activity in PD-1^{hi} CXCR5^{-} CCR2^{-}, PD-1^{hi} CXCR5^{-} CCR2^{+}, and PD-1^{hi} CXCR5^{+} cells (**Fig. 4b,c**). PD-1^{hi} CXCR5^{-} cells from synovial fluid and blood also enhanced IgG production in the co-cultures (**Fig. 4d**). Neutralization of IL-21 inhibited plasma cell differentiation induced by both blood PD-1^{hi} CXCR5^{+} cells and PD-1^{hi} CXCR5^{-} cells by ~90% (**Fig. 4e**). Expression of SLAMF5, a factor important for T-B interactions,[3] was elevated on both PD-1^{hi} CXCR5^{-} and PD-1^{hi} CXCR5^{+} cells, and antibody blockade of SLAMF5, but not SLAMF6, completely abrogated plasma cell differentiation and IgG production (**Fig. 4f**, **Extended Data Fig. 6a-c**). Consistent with a link *in vivo*, RA treatment escalation reduced the frequency of circulating plasmablasts in parallel with the reduction in PD-1^{hi} CXCR5^{-} T cells (**Fig. 1j**).

Finally, we evaluated the localization of PD-1^{hi} T cells in RA synovium by immunofluorescence microscopy. CD3^{+} T cells with bright PD-1 staining were readily identified (**Fig. 4g**). CXCR5 appeared on CD20^{+} B cells and on a minority of PD-1^{hi} T cells that were enriched within lymphoid aggregates **(Fig. 4h,i)**.

However, PD-1^{hi} CXCR5^{-} cells outnumbered PD-1^{hi} CXCR5^{+} cells within lymphoid aggregates and were ~4-fold more abundant than PD-1^{hi} CXCR5^{+} cells in regions outside of lymphoid aggregates (**Fig. 4i**). Within lymphoid aggregates, both PD-1^{hi} CXCR5^{-} cells and PD-1^{hi} CXCR5^{+} cells were found adjacent to B cells (**Fig. 4h,j**). However, in areas outside of lymphoid aggregates, the majority of PD-1^{hi} cells adjacent to B cells were CXCR5^{-} (**Fig. 4j, Extended Data Fig. 6d**)**.** These results suggest a unique capacity of PD-1^{hi} CXCR5^{-} T cells to interact with B cells both within lymphoid aggregates and more diffusely throughout the inflamed synovium.

Here we have defined a PD-1^{hi} CXCR5^{-} CD4^{+} Tph cell population markedly expanded in rheumatoid arthritis that combines B cell helper function with a migratory program targeting inflamed tissues. The abundance of Tph cells in RA synovium highlights the importance of tissue-localized T-B cell interactions[22]. Tph cells may infiltrate chronically inflamed tissues, which would not be expected to readily recruit Tfh cells, providing a potential mechanism for the initiation of ectopic lymphoid structures[23-25]. Tph cell production of CXCL13 and IL-21 may recruit both Tfh and B cells, promoting local autoantibody production that may not be reflected in serum, and perhaps modulating other B cell functions such as cytokine production[7,26-28]. Identification of the Tph cell phenotype considerably expands the spectrum of B cell-helper T cells that may be assessed as biomarkers for autoantibody-associated diseases. Further, high expression of

PD-1 on Tph cells may offer a potential strategy for therapeutic targeting of tissue T cell-B cell interactions.

REFERENCES

1 Maecker, H. T., McCoy, J. P. & Nussenblatt, R. Standardizing immunophenotyping for the Human Immunology Project. *Nature reviews. Immunology* **12**, 191-200, doi:10.1038/nri3158 (2012).

2 McInnes, I. B. & Schett, G. The pathogenesis of rheumatoid arthritis. *The New England journal of medicine* **365**, 2205-2219, doi:10.1056/NEJMra1004965 10.7748/phc2011.11.21.9.29.c8797 (2011).

3 Cannons, J. L. *et al.* Optimal germinal center responses require a multistage T cell:B cell adhesion process involving integrins, SLAM-associated protein, and CD84. *Immunity* **32**, 253-265, doi:10.1016/j.immuni.2010.01.010 (2010).

4 Crotty, S. Follicular helper CD4 T cells (TFH). *Annual review of immunology* **29**, 621-663, doi:10.1146/annurev-immunol-031210-101400 (2011).

5 Amir el, A. D. *et al.* viSNE enables visualization of high dimensional single-cell data and reveals phenotypic heterogeneity of leukemia. *Nature biotechnology* **31**, 545-552, doi:10.1038/nbt.2594 (2013).

6 Takemura, S. *et al.* Lymphoid neogenesis in rheumatoid synovitis. *Journal of immunology* **167**, 1072-1080 (2001).

7 Humby, F. *et al.* Ectopic lymphoid structures support ongoing production of class-switched autoantibodies in rheumatoid synovium. *PLoS medicine* **6**, e1, doi:10.1371/journal.pmed.0060001 (2009).

8 Wherry, E. J. & Kurachi, M. Molecular and cellular insights into T cell exhaustion. *Nature reviews. Immunology* **15**, 486-499, doi:10.1038/nri3862 (2015).

9 Kamphorst, A. O. & Ahmed, R. Manipulating the PD-1 pathway to improve immunity. *Current opinion in immunology* **25**, 381-388, doi:10.1016/j.coi.2013.03.003 (2013).

10 Johnston, R. J. *et al.* Bcl6 and Blimp-1 are reciprocal and antagonistic regulators of T follicular helper cell differentiation. *Science* **325**, 1006-1010, doi:10.1126/science.1175870 (2009).

11 Kroenke, M. A. *et al.* Bcl6 and Maf cooperate to instruct human follicular helper CD4 T cell differentiation. *Journal of immunology* **188**, 3734-3744, doi:10.4049/jimmunol.1103246 (2012).

12 DeGrendele, H. C., Estess, P. & Siegelman, M. H. Requirement for CD44 in activated T cell extravasation into an inflammatory site. *Science* **278**, 672-675 (1997).

13 Forster, R. *et al.* CCR7 coordinates the primary immune response by establishing functional microenvironments in secondary lymphoid organs. *Cell* **99**, 23-33 (1999).

14 Picelli, S. *et al.* Smart-seq2 for sensitive full-length transcriptome profiling in single cells. *Nature methods* **10**, 1096-1098, doi:10.1038/nmeth.2639 (2013).

15 Chtanova, T. *et al.* T follicular helper cells express a distinctive transcriptional profile, reflecting their role as non-Th1/Th2 effector cells that provide help for B cells. *Journal of immunology* **173**, 68-78 (2004).

16 Locci, M. *et al.* Human circulating PD-1+CXCR3-CXCR5+ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. *Immunity* **39**, 758-769, doi:10.1016/j.immuni.2013.08.031 (2013).
17 Weinstein, J. S. *et al.* Global transcriptome analysis and enhancer landscape of human primary T follicular helper and T effector lymphocytes. *Blood* **124**, 3719-3729, doi:10.1182/blood-2014-06-582700 (2014).
18 Kenefeck, R. *et al.* Follicular helper T cell signature in type 1 diabetes. *The Journal of clinical investigation* **125**, 292-303, doi:10.1172/JCI76238 (2015).
19 Kuziel, W. A. *et al.* Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2. *Proceedings of the National Academy of Sciences of the United States of America* **94**, 12053-12058 (1997).
20 Rot, A. & von Andrian, U. H. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. *Annual review of immunology* **22**, 891-928, doi:10.1146/annurev.immunol.22.012703.104543 (2004).
21 Langenkamp, A. *et al.* Kinetics and expression patterns of chemokine receptors in human CD4+ T lymphocytes primed by myeloid or plasmacytoid dendritic cells. *European journal of immunology* **33**, 474-482, doi:10.1002/immu.200310023 (2003).
22 Vu Van, D. *et al.* Local T/B cooperation in inflamed tissues is supported by T follicular helper-like cells. *Nature communications* **7**, 10875, doi:10.1038/ncomms10875 (2016).
23 Pitzalis, C., Jones, G. W., Bombardieri, M. & Jones, S. A. Ectopic lymphoid-like structures in infection, cancer and autoimmunity. *Nature reviews. Immunology* **14**, 447-462, doi:10.1038/nri3700 (2014).
24 Kobayashi, S. *et al.* A distinct human CD4+ T cell subset that secretes CXCL13 in rheumatoid synovium. *Arthritis and rheumatism* **65**, 3063-3072, doi:10.1002/art.38173 (2013).
25 Manzo, A. *et al.* Mature antigen-experienced T helper cells synthesize and secrete the B cell chemoattractant CXCL13 in the inflammatory environment of the rheumatoid joint. *Arthritis and rheumatism* **58**, 3377-3387, doi:10.1002/art.23966 (2008).
26 Scheel, T., Gursche, A., Zacher, J., Haupl, T. & Berek, C. V-region gene analysis of locally defined synovial B and plasma cells reveals selected B cell expansion and accumulation of plasma cell clones in rheumatoid arthritis. *Arthritis and rheumatism* **63**, 63-72, doi:10.1002/art.27767 (2011).
27 Shen, P. & Fillatreau, S. Antibody-independent functions of B cells: a focus on cytokines. *Nature reviews. Immunology* **15**, 441-451, doi:10.1038/nri3857 (2015).
28 Yeo, L. *et al.* Expression of FcRL4 defines a pro-inflammatory, RANKL-producing B cell subset in rheumatoid arthritis. *Annals of the rheumatic diseases* **74**, 928-935, doi:10.1136/annrheumdis-2013-204116 (2015).
29 Finck, R. *et al.* Normalization of mass cytometry data with bead standards. *Cytometry. Part A : the journal of the International Society for Analytical Cytology* **83**, 483-494, doi:10.1002/cyto.a.22271 (2013).

30 Finak, G., Perez, J. M., Weng, A. & Gottardo, R. Optimizing transformations for automated, high throughput analysis of flow cytometry data. *BMC bioinformatics* **11**, 546, doi:10.1186/1471-2105-11-546 (2010).

31 Bray, N. P., H.; Melsted, P.; Pachter, L. . Near-optimal RNA-Seq quantification. *arXiv* **1505**, 02710v02712 (2015).

1.

Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

Acknowledgements

This work was supported by T32 AR007530-31 and the William Docken Inflammatory Autoimmune Disease Fund (to M.B.B), Mallinckrodt Research Fellowship (to D.A.R), R01 AR064850-03 (to Y.C.L), NIH 5U01GM092691-05, 1U19 AI111224-01 and Doris Duke Charitable Foundation Grant #2013097 (to S.R.), Rheumatology Research Foundation's Scientist Development Award (to L.A.H.), K01 AR066063 (to L.T.D.), Arthritis Research UK programme grant #19791 (to C.D.B), and Arthritis Research UK clinician scientist fellowship #18547 (to A.F). J.L.M was supported by the FP7-HEALTH-F2-2012-305549 EuroTEAM. P.A.N. was supported by the Fundación Bechara. We thank Adam Chicoine and the BWH Human Immunology Center Flow Cytometry Core for cell sorting assistance.

Author Contributions

D.A.R conceived of the project, performed experiments, analyzed data, and wrote the manuscript. M.F.G, Y.L, N.T., and F.M. performed experiments and analyzed data. K.S. analyzed the RNA sequencing data. C.F. analyzed mass cytometry data. J.L.M. performed the immunofluorescence microscopy. J.A.L. developed reagents and assisted with mass cytometry. K.W., L.A.H., P.A.N., M.E.W., Y.C.L., J.S.C., D.J.T., E.M.M., S.M.H., L.T.D., V.P.B., L.B.I., S.M.G., A.B.P., A.F., and C.D.B participated in study design, patient recruitment, sample acquisition, and review of the data. S.R. co-supervised the project, analyzed data, and co-wrote the manuscript. M.B.B. conceived of the project, supervised the work, analyzed data, and co-wrote the manuscript. All authors discussed the results and commented on the manuscript.

Author Information

RNA sequencing data is available at the GEO repository, accession number GSE80253. Reprints and permissions information is available at www.nature.com/reprints. The authors have no competing financial interests. Correspondence and requests for materials should be addressed to M.B.B. (mbrenner@research.bwh.harvard.edu) or D.A.R. (darao@partners.org).

	Synovial Panel		Blood cell Panel	
Metal	Target	Clone	Target	Clone
89Y	CD45	HI30		
103Rh			Live/Dead	Cell-ID
141Pr	CD27	M-T271	CD27	M-T271
142Nd	CD19	HIB19	CD45RA	HI100
143Nd	RANKL	MIH24	CD44	BJ18
144Nd	CD64	10.1	CD39	A1
145Nd	CD16	3G8	CD16	3G8/B73.1
146Nd	CD8α	RPA T8	CD8α	RPA T8
147Sm	FAP	Poly	CD45RO	UCHL1
148Nd	CD20	2H7	CD28	CD28.2
149Sm	CD45RO	UCHL1	CD25	M-A251
150Nd	CD38	HIT2		
151Eu	PD-1	EH12.2H7	PD-1	EH12.2H7
152Sm	CD14	M5E2		
153Eu	CD69	FN50	CD69	FN50
154Sm	CXCR5	J252D4	CXCR5	J252D4
155Gd	CD4	RPA T4	CD4	RPA T4
156Gd	Podoplanin	NC-08	CD73	AD2
158Gd	CD3	UCHT1	CD3	UCHT1
159Tb	CD11c	Bu15	CD57	HCD57
160Gd	FcRL4	413D12	ICOS	C398.4A
161Dy	CD138	MI15		
162Dy	CD90	5E 10	CXCR3	G025H7
163Dy	CCR2	K036C2		
164Dy	Cadherin11	23C6	CD161	HP-3G10
165Ho	FoxP3	PCH101	FoxP3	PCH101
166Er	CD34	581		
167Er	CD146	SHM-57	CD38	HIT2
168Er	IgA	9H9H11	CCR6	G034E3
169Tm	TCRγδ	B1	CCR7	G043H7
170Er	ICOS	C398.4A		
171Yb	CD66b	G10F5	CD127	A019D5
172Yb	IgM	MHM-88	CD122	TU27
173Yb	CD144	BV9	TIGIT	MBSA43
174Yb	MHCII	L243	HLA-DR	L243
175Lu	IgD	IA6-2	Tbet	4B10
176Yb	VCAM-1	STA	Perforin	dG9
195Pt	Live/Dead	Cell-ID		

Extended Data table 1. Mass cytometry panels for analysis of synovial and blood cells

	Patient	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10
Synovial Tissue Donors	**Age**	57	54	76	46	46	79	62	63	52	43
	Sex	F	F	F	F	F	F	M	M	F	F
	Disease Duration (yrs)	13	17	4	8	19	0.5	19	8	N/A	N/A
	CDAI	14	9	17	15	21	25	5	9	N/A	N/A
	CRP (mg/L)	25	8	8	11	17	19	13	66	76	0.8
	Methotrexate	No	Yes	No	No	No	No	No	Yes	No	No
	Biologic therapy	Yes	Yes	Yes	Yes	Yes	No	No	No	No	Yes
	Other synthetic DMARD	Yes	No	Yes	No	Yes	No	No	No	Yes	No

Samples from patients 1-6 were used for mass and flow cytometry phenotyping.
Samples from patients 7-10 were also included in flow cytometry phenotyping.

		Control	Seropositive RA	Seronegative RA	SpA
Blood Cross-sectional Cohort	**Number**	35	42	16	11
	Age	61 ± 13	58 ± 14	58 ± 13	48 ± 12
	Female	22 (63)	33 (78)	11 (69)	5 (45)
	Disease Duration (yrs)	N/A	13 ± 9	14 ± 10	10 ± 6
	C-reactive protein (mg/L)	ND	9.3 ± 17.4	6.3 ± 8.5	3.9 ± 4.2
	CDAI	ND	13.7 ± 8.1	9.8 ± 7.6	ND
	Methotrexate	0	19 (45)	8 (50)	2 (18)
	Anti-TNF	0	16 (38)	6 (38)	10 (90)
	Other biologics	0	10 (24)	5 (31)	0
	Other synthetic DMARDs	0	4 (10)	1 (6)	0

Average ± SD are shown. Parentheses indicate proportion of patients. Other biologics include abatacept, rituximab, tocilizumab, tofacitinib.

		Improved	Not Improved
Blood Longitudinal Cohort	**Number**	18	5
	Age	49 ± 17	57 ± 10
	Female	17 (94)	4 (80)
	CDAI Before	17.6 ± 9.3	21.7 ± 8.9
	CDAI After	6.3 ± 4.2	25.6 ± 10.2
	Started methotrexate	7	4
	Started anti-TNF	4	0
	Started other biologic	7	1

Average ± SD are shown. Parentheses indicate proportion of patients.

Extended Data table 2. Clinical characteristics of evaluated patients.

Target	Clone	Fluorophore
CD27	TB01	FITC
CXCR3	CEW33D	PE
CD4	RPA-T4	PE-Cy7
ICOS	ISA-3	PerCP-Cy5.5
CXCR5	J252D4	BV421
CD45RA	HI100	BV510
HLA-DR	G46-6	BV605
CD49d	9F10	BV711
PD-1	EH12.2H7	APC
CD3	HIT3A	AlexaFluor700
CD29	TS2/16	APC-Cy7
Live/Dead		Propidium iodide

Extended Data table 3. Flow cytometry panel for identifying PD-1^{hi} cells

Gene	logFC PD-1$^-$ Vs PD-1^{hi}	p-value	adjusted p-value
PD-1	-6.394163674	1.03E-17	2.07E-13
TOX	-3.973939225	7.21E-13	7.21E-09
ITM2A	-1.206121015	4.54E-10	3.02E-06
TIGIT	-1.919479399	1.03E-09	5.15E-06
MAF	-1.424142776	4.43E-09	1.77E-05
CA6	3.052456565	6.13E-09	2.04E-05
CST7	-3.162619611	1.47E-08	3.80E-05
SCML1	3.900002703	1.71E-08	3.80E-05
SCO2	-5.202859453	1.67E-08	3.80E-05
CDCA7	-4.502988841	2.56E-08	5.12E-05
RAB37	-1.574133208	6.90E-08	0.000115075
ICA1	-2.953123584	2.32E-07	0.000323551
EZH2	-3.019907996	2.43E-07	0.000323551
GZMK	-2.753052708	2.68E-07	0.0003348
MAP3K9	-2.362184122	4.44E-07	0.00052219
PFN1	-1.46238995	7.05E-07	0.000704904
SLAMF6	-1.241364005	8.88E-07	0.000817585
EPSTI1	-2.040445226	8.99E-07	0.000817585
NEFL	4.046814543	1.14E-06	0.00098964
CHN1	-3.450144917	1.22E-06	0.001005063
UBE2L6	-1.228575119	1.55E-06	0.001144629
FANCI	-2.790697425	1.77E-06	0.001264215
PSMA4	-1.420908253	2.22E-06	0.001482222
TOX2	-3.637770018	2.72E-06	0.001698657
FABP5	-2.439672325	3.07E-06	0.001806205
ANXA2	-1.269580271	3.38E-06	0.001932684
CTLA4	-1.739000105	4.31E-06	0.002330776
PLAG1	3.599551478	4.77E-06	0.002510472
HVCN1	-3.516538014	5.08E-06	0.002605277
FAM210A	-2.782673649	5.37E-06	0.002683965
ALOX5	3.436967858	5.88E-06	0.002799526
RGS1	-1.204580998	6.09E-06	0.002834562
MYL6B	-3.072692168	8.27E-06	0.003651008
CEP128	-3.16366403	8.18E-06	0.003651008
ENC1	-3.605191323	8.40E-06	0.003651008
MIS18BP1	-2.065611325	8.89E-06	0.003784483
F5	-1.463762553	1.00E-05	0.004049502
FN1	2.504688725	1.07E-05	0.004049502
CXCR3	-3.01503276	1.06E-05	0.004049502
ASB13	3.35115795	1.06E-05	0.004049502
HIST2H2BF	3.678067821	9.85E-06	0.004049502

PRR5L	-2.171927479	1.10E-05	0.004078013
KRT72	3.194468773	1.24E-05	0.004427712
BZRAP1	-1.970565679	1.37E-05	0.004807752
DUSP2	-1.459107208	1.55E-05	0.005330045
DHFR	-2.732987087	1.74E-05	0.005621497
FBXO41	-2.405412912	1.94E-05	0.00614422
CCDC86	-3.430212114	1.99E-05	0.00620599
FCRL3	-1.770956334	2.06E-05	0.00627086
AKR1C3	-3.308816301	2.07E-05	0.00627086
SHMT2	-1.534205662	2.17E-05	0.006375986
DDX54	-1.733666484	2.21E-05	0.006411258
UBE2A	-1.316870902	2.88E-05	0.007682071
ANXA9	2.915422557	2.85E-05	0.007682071
TUBB4B	-1.239062048	3.12E-05	0.008096696
TIMELESS	-2.607726722	3.24E-05	0.008255379
CCL5	-3.355363898	3.26E-05	0.008255379
UQCRC1	-1.286406184	3.42E-05	0.008442364
TBC1D4	-1.300326318	3.49E-05	0.008514635
SYT11	-1.382472289	3.88E-05	0.009230883
PMAIP1	-2.193153793	3.84E-05	0.009230883
DIRC2	-2.784076776	4.00E-05	0.009304666
SOX8	1.93871557	4.26E-05	0.009799307
SPG20	1.801986978	4.50E-05	0.009899027
DPP3	-1.957767214	4.39E-05	0.009899027
DUSP4	-2.502643153	4.50E-05	0.009899027

Extended Data table 4. Significantly differentially expressed genes between PD-1^{-} and PD-1^{hi} cells

Gene	logFC PD-1^{hi} CXCR5^{-} vs PD-1^{hi} CXCR5^{+}	p-value	adjusted p-value
RPL39	-1.234532087	1.42E-07	0.000230324
LSP1	1.200685645	2.65E-07	0.00029438
RPL34	-1.273114838	7.88E-07	0.000477468
TTC4	4.631494115	1.29E-06	0.00063025
LIME1	1.562056436	2.07E-06	0.000920442
CCR2	5.094906534	2.25E-06	0.000957805
ACTN4	2.323677525	2.83E-06	0.001130754
CTSH	2.649046873	3.19E-06	0.001251255
PLAC8	-1.351296215	6.85E-06	0.002360784
GLIPR2	1.396871219	7.14E-06	0.00237986
PRR5	2.819632034	1.02E-05	0.003057343
RGS19	2.456286463	1.19E-05	0.003446459
SAMD3	1.24882546	2.50E-05	0.006505926
FOS	-1.595174448	3.68E-05	0.008767004
ANXA4	2.266594524	4.03E-05	0.009370528
LTK	3.888867594	4.20E-05	0.009656997

Extended Data table 5. Significantly differentially expressed genes between PD-1^{hi} CXCR5^{-} cells and PD-1^{hi} CXCR5^{+} cells

METHODS

Human subjects research

Human subjects research was performed according to the Institutional Review Boards at Partners HealthCare, Hospital for Special Surgery, or the University of Birmingham Local Ethical Review Committee (Birmingham, UK) via approved protocols with appropriate informed consent as required. Patients with RA fulfilled the ACR 2010 Rheumatoid Arthritis classification criteria. Rheumatoid factor and anti-CCP antibody status, C-reactive protein level, and medication usage were obtained by review of electronic medical records. Biologic therapy was defined as use of anti-TNF, abatacept, rituximab, tocilizumab, or tofacitinib. Synovial tissue samples for mass and flow cytometry were collected from seropositive RA patients undergoing arthroplasty at the Hospital for Special Surgery, New York or at Brigham and Women’s Hospital, Boston. Samples with lymphocytic infiltrates on histology were prioritized for analyses. Synovial tissue for microscopy was acquired by synovial biopsy of a clinically inflamed joint from seropositive RA patients within the Birmingham early arthritis cohort (BEACON) at the University of Birmingham, UK.

Synovial fluid samples were obtained as excess material from a separate cohort of patients undergoing diagnostic or therapeutic arthrocentesis of an inflammatory knee effusion as directed by the treating rheumatologist. These samples were de-identified; therefore, additional clinical information was not available, except for the 3 patients from whom paired synovial fluid and blood were obtained.

Blood samples for clinical phenotyping were obtained from patients seen at the Brigham and Women's Hospital Arthritis Center. For blood cell analyses in the cross-sectional cohort, CDAI was measured by the treating clinician on the day of sample acquisition. Anti-CCP titers were measured using the Immunoscan CCPLus ELISA (Eurodiagnostica), with a positive result defined as >25 units/mL. For RA patients followed longitudinally, a new treatment was initiated at the discretion of the treating physician, and CDAIs were determined at each visit by trained research study staff. Blood samples were acquired before initiation of a new biologic therapy or within 1 week of starting methotrexate. Concurrent prednisone at doses <10mg/day were permitted.

All synovial fluid and blood samples were subjected to density centrifugation using Ficoll-Hypaque to isolate mononuclear cells, which were cryopreserved for batched analyses. Most phenotypic and transcriptomic analyses of blood T cells were performed on samples from both RA patients and non-inflammatory controls, with similar results unless specifically indicated. *In vitro* PD-1^{hi} T cell interconversion assays and *in vitro* B cell helper-assays using blood T cells were performed using PBMC from blood bank leukoreduction collars from anonymous donors.

All blood $CD4^+$ T cell analyses included only $CD45RA^-$ memory $CD4^+$ T cells except where naïve ($CD45RA^+$) cells are specifically indicated. Here the term 'memory' is used to denote an 'antigen-experienced' status indicated by loss of the naïve T cell marker CD45RA. This population includes both resting and activated antigen-experienced T cells. Synovial fluid and tissue analyses also utilize only memory $CD4^+$ T cells unless total $CD4^+$ T cells are indicated. Naïve T cells constituted <10% of the total population of $CD4^+$ T cells in synovial tissue and synovial fluid.

Synovial tissue analysis

Synovial samples were acquired from discarded arthroplasty tissue. Synovial tissue was isolated by careful dissection, minced, and digested with 100µg/mL LiberaseTL and 100µg/mL DNaseI (both Roche) in RPMI (Life Technologies) for 15 minutes, inverting every 5 minutes. Cells were passed through a 70µm cell strainer, washed, subjected to red blood cell lysis, and cryopreserved in Cryostor CS10 (BioLife Solutions) for batched analyses.

Mass cytometry

Cryopreserved disaggregated synovial cells or PBMCs were thawed into RPMI + 10% FBS (HyClone). Viability was assessed with rhodium for PBMCs and cisplatin (both Fluidigm) for synovial cells. Cells were then washed and stained with primary antibody cocktails at 1:100 dilution (Extended Data Table 4). All antibodies were obtained from the Longwood Medical Area CyTOF Antibody

Resource Core (Boston, MA). Cells were then washed, fixed and permeabilized using the Ebioscience Transcription Factor Fix/Perm Buffer for 45 minutes, washed in PBS/1%BSA/0.3% saponin, then stained for intracellular markers. Cells were re-fixed in formalin (Sigma), washed with Milli-Q water, and analyzed on a CyTOF2 for PBMC or Helios (Fluidigm) for synovial cells. Mass cytometry data were normalized using EQ™ Four Element Calibration Beads (Fluidigm) as described[29].

viSNE analyses were performed on cytometry data from 3 of 6 synovial tissue samples, 3 of 9 synovial fluid samples, and 8 of 14 blood samples using the Barnes-Hut SNE implementation on Cytobank (www.cytobank.org). All 3 individual synovial tissue sample analyses are shown. For synovial fluid and blood cell analyses, one representative patient sample is shown. For synovial tissue mass cytometry data, gated $CD4^+$ T cells were analyzed using all available protein markers, and each synovial tissue sample was analyzed individually to allow for maximal resolution. For paired synovial fluid-blood flow cytometry data, gated memory $CD4^+$ T cells from synovial fluid and blood were analyzed together in a single viSNE analysis for direct comparison using an equal number of randomly selected cells from each sample. For blood mass cytometry analyses, equal numbers of gated memory $CD4^+$ T cells from each sample were analyzed together using all markers except those used for gating (CD3, CD4, CD45RO). Comparison of marker expression on PD-1^{hi} $CXCR5^-$ and PD-1^{hi} $CXCR5^+$ cells was performed with R-3.2 using permutation Wilcoxon rank-sum tests adjusted for multiple testing. For graphical representation of differential expression, mass cytometry data were transformed using the inverse hyperbolic sine[30].

Flow cytometry and cell sorting

For phenotypic analyses, cryopreserved cells were thawed into warm RPMI/10% FBS, washed once in cold PBS, and stained in PBS/1% BSA with antibody mixes as in Extended Data Table 2 for 45 minutes. Additional antibodies used include SLAM-AF488 (A12), SLAMF5-PE (CD84.1.21), SLAMF6-PE (NT-7), CCR2-PE (K036C2), CX3CR1-FITC (2A9-1), CD38-PE (HIT2), CD138-PE/Cy7 (MI15), CTLA4-PerCP/Cy5.5 (L3D10) from BioLegend, CCR5-FITC (2D7) and FoxP3-AF647 (236A/E7) from BD Biosciences, LAG-3-APC from R&D Systems, TIM-3-PE/Cy7 (F38-2E2) and TIGIT-PE (MBSA43) from eBioscience.

Cells were washed in cold PBS, passed through a 70-micron filter, and data acquired on a BD FACSAria Fusion, BD Fortessa, or BD Canto II analyzer using FACSDiva software. Data were analyzed using FlowJo 10.0.7. For blood cell quantification in Figure 2, samples were analyzed in uniformly processed batches of coded samples with multiple disease conditions included in each batch. Upon data acquisition, disease categories were assigned to data files. A single set of gates for PD-1, CXCR5, ICOS, and MHC II was applied to all samples. The percentage of PD-1^{hi} T cell populations among memory CD4^{+} T cells populations and the percentage of plasmablasts (CD19^{+} CD20^{lo} CD38^{hi} CD27^{+}) among total CD19^{+} B cells were calculated for indicated samples.

T cells were sorted directly from synovial fluid and synovial tissue samples. For sorting blood T cells, total $CD4^+$ T cells were first isolated by magnetic bead negative selection (Miltenyi Biotec). Cell sorting was performed on a BD FACSAria Fusion sorter using a 70 micron nozzle. Sort gates were drawn as depicted in **Extended Data Fig. 1d**. Cell purity was routinely >98%. For functional analyses, cells were sorted into cold RPMI/10% FBS. For RNA analyses, sorted cells were lysed in RLT lysis buffer (Qiagen) with 1% β-mercaptoethanol (Sigma)**.**

Intracellular cytokine staining

Synovial fluid mononuclear cells were stained with anti-PD-1-PE/Dazzle 594, CXCR5-BV605, and CD4-BV650 (Biolegend), and propidium iodide. $CXCR5^-$ PD-1^{hi}, PD-1^{int}, and PD-1^- $CD4^+$ T cells sorted as above were pelleted by centrifugation and resuspended in RPMI/10% FBS at a density of $5x10^5$ cells/mL in 24-well plates. Cells were stimulated with either anti-CD3/anti-CD28 beads at a ratio of 2:1 (cell:bead) for 24 hours, or with PMA (50ng/mL) and ionomycin (1μg/mL). Brefeldin A and monensin (both 1:1000, eBioscience) were added for the last 5 hours. Cells were washed twice in cold PBS, incubated for 30 minutes with Fixable Viability Dye eFluor 455UV (eBioscience), washed in PBS/1%BSA, and then fixed and permeabilized using the eBioscience Transcription Factor Fix/Perm Buffer. Cells were washed in PBS/1%BSA/0.3% saponin and incubated with IL-21-APC (3A3-N2), IL-2-PE/Cy7 (MQ1-17H12), and CXCL13-AF700

(53610, R&D Systems) for 30 minutes, washed once, filtered, and data acquired on a BD Fortessa analyzer.

Intracellular transcription factor staining

Synovial tissue and synovial fluid cells were thawed, washed twice in PBS, and incubated with Fixable Viability Dye eFluor 455UV (eBioscience) for 30 minutes. Cells were then washed in PBS/1%BSA and stained with antibodies against surface markers anti-CD3-AF700, anti-CD4-BV650, anti-CCR2-PE, anti-CXCR5-BV421, anti-PD-1-PE/Dazzle 594 (all Biolegend) for 30 minutes. Cells were washed once and incubated with eBioscience Transcription Factor Fix/Perm Buffer. Cells were washed in PBS/1%BSA/0.3% saponin and incubated in intracellular antibodies anti-MAF-PerCP-eFluor710 (sym0F1, eBioscience), anti-Bcl6-APC (BCL-UP, eBioscience), and anti-Blimp-1-AF488 (646702, R&D Systems) at 1:20 dilutions for 4 hours. Cells were washed once, filtered, and data acquired on a BD Fortessa analyzer. Intracellular detection of FoxP3 and CTLA-4 were performed by the same method on magnetic-bead purified blood $CD4^{+}$ T cells using the indicated surface markers.

RT-PCR analyses

RNA isolated using RNeasy Micro Kits (Qiagen). cDNA was prepared using Quantitect RT-PCR (Qiagen) and PCR performed with Brilliant III SYBRGreen on an a Stratagene Mx3000. Primers used were as follows: RPL13A (Forward: 5’-CATAGGAAGCTGGGAGCAAG-3’; Reverse: 5’-GCCCTCCAATCAGTCTTCTG-3’), IL-2

(Forward: 5’-AGAACTCAAACCTCTGGAGGAAG-3’; Reverse: 5’-GCTGTCTCAGCATATTCACAC-3’), IFN-γ (Forward: 5’-GCATCGTTTTGGGTTCTCTTG-3’; Reverse: 5’-AGTTCCATTATCCGCTACATCTG-3’), IL-10 (Forward: 5’-CGCATGTGAACTCCCTGG-3’; Reverse: 5’-TAGATGCCTTTCTCTTGGAGC-3’), IL-21 (Forward: 5’-AGGAAACCACCTTCCACAAA-3’; Reverse: 5’-GAATCACATGAAGGGCATGTT-3’), CXCL13 (Forward: 5’-TCTCTGCTTCTCATGCTGCT-3’; Reverse: 5’-TCAAGCTTGTGTAATAGACCTCCA-3’), PD-1 (Forward: 5’-CCAGGATGGTTCTTAGACTCC-3’; Reverse: 5’-TTTAGCACGAAGCTCTCCGAT-3’), CXCR5 (Forward: 5’-GGGAGCCTCTCAACATAAGAC-3’; Reverse: 5’-CCAATCTGTCCAGTTCCCAGA-3’), MAF (Forward: 5’-CCGTCCTCTCCCGAGTTTTT-3’; Reverse: 5’-TGCTGGGGCTTCCAAAATGT-3’), Bcl6 (Forward: 5’-GTTTCCGGCACCTTCAGACT-3’; Reverse: 5’-CTGGCTTTTGTGACGGAAAT-3’), BATF (Forward: 5’-TGGCAAACAGGACTCATCTG-3’; Reverse: 5’-CTGTTTCTCCAGGTCTTCGC-3’), SAP (Forward: 5’-GCTATTTGCTGAGGGACAGC-3’; Reverse: 5’-TGTCTGGGACACTCGGTATG-3’), Blimp-1 (Forward: 5’-AACTTCTTGTGTGGTATTGTCGG-3’; Reverse: 5’-TCTCAGTGCTCGGTTGCTTT-3’). Expression levels relative to control gene RPL13A were calculated.

RNA sequencing

RNA was isolated from 800-1000 cells from sorted T cell subpopulations as described. 5uL of total RNA were placed in wells of a 96-well plate and RNA sequencing libraries were prepared at Broad Technology Labs at the Broad Institute of Harvard and MIT using the Illumina SmartSeq2 platform. Samples were sequenced on a NextSeq500 using 75bp paired-end reads to an average depth of 9M pairs of reads per sample. All cDNA transcripts from Ensembl release 82 were quantified with Kallisto version 0.42.4[31]. We used limma to model each gene as a linear combination of donor-specific effects. The residuals from these models were tested by ANOVA across 8 gates, and 581 genes with a significant F statistic with <5% FDR were selected for PCA. Heatmaps show row-normalized relative gene expression z-scores across columns (mean 0 and variance 1), with subpopulations of PD-1^{hi} CXCR5^{-} or PD-1^{hi} CXCR5^{+} averaged to yield overall PD-1^{hi} CXCR5^{-} and PD-1^{hi} CXCR5^{+} expression values. In comparisons of specific cell populations, genes with log fold change >1.2 and FDR <1% were considered differentially expressed.

PD-1^{hi} cell *in vitro* stimulation assays

CD4^{+} T cells were purified from PBMCs from leukoreduction collars by magnetic bead negative selection and stained with anti-CD4-BV650, anti-CD45RA-BV510, anti-PD-1-APC, anti-CXCR5-BV605, and anti-CCR2-PE/Cy7. Naïve CD4^{+} T cells and memory CD4^{+} T cell subpopulations were sorted into RPMI/10% FBS. 50,000 cells were resuspended in RPMI/10%FBS at 0.25 x 10^{6} cells/mL and cultured with anti-CD3/CD28 beads (Dynabeads) at a cell:bead ratio of 5:1 for 2 or 7 days. Cells were then either re-stained with anti-PD-1-PE and anti-CXCR5-BV421 antibodies and sorted into lysis buffer for RT-PCR analyses, or stained with CCR2-PE and CXCR5-BV421 and analyzed by intracellular flow cytometry for transcription factors as above.

T cell-B cell co-cultures

Total B cells were isolated first from PBMCs from blood bank leukoreduction collars by magnetic bead positive selection using CD19 (Miltenyi), then $CD4^+$ T cells were isolated by negative selection. B cells were stained with CD14-APC, CD3-PeCy7, and CD27-BV510 (all from Biolegend), and memory B cells sorted as $CD27^+$ $CD14^-$ $CD3^-$ cells on a BD FACSAria Fusion to remove contaminating T cells and monocytes. Sorted T cell populations were co-cultured with autologous memory B cells at a ratio of 1:10 in 100uL of RPMI/10%FBS and stimulated with LPS (5µg/mL) and SEB (1µg/mL) for 7 days. For co-cultures using synovial tissue or synovial fluid T cells, allogeneic memory B cells from PBMC were used. Supernatants were collected and total IgG measured by ELISA (eBioscience). Cells were harvested and analyzed by flow cytometry, with plasmablasts defined as $CD19^+$ $CD20^{low}$ $CD38^{hi}$ $CD27^+$ and plasma cells defined as $CD19^+$ $CD20^{low}$ $CD38^{hi}$ $CD27^+$ $CD138^+$. For blocking experiments, 10µg/ml anti-SLAMF5 or anti-SLAMF6 antibodies (Biolegend) or 20µg/mL IL-21R-Ig (R&D Systems) were used.

Immunofluorescence microscopy

6 micron sections of synovium frozen in OCT were fixed in acetone, rehydrated in PBS, and blocked with 10% normal goat serum prior to application of primary antibodies as follows: PD-1 (EH12.2H7, BioLegend), CD3 (SP7, Abcam), CD20 (L26, Dako), CXCR5 (MAB190, R&D Systems), all at a dilution of 1:100 except

for CD20, which was used at 1:300. All secondary antibodies were raised in goat. CXCR5 was detected using anti mouse IgG2b biotin (Southern biotech) followed by streptavidin conjugated AlexaFluor 546 (Life Technologies), CD20 with anti-mouse IgG2a FITC (both Southern Biotech), PD-1 with anti-mouse IgG1 conjugated to AlexaFluor 647 and CD3 with anti-rabbit AlexaFluor 546 (both Life Technologies). FITC staining was amplified with anti-FITC AlexaFluor 488 (Life Technologies). Slides were mounted using ProLong Diamond (Life Technologies), left to cure overnight and imaged using a Zeiss LSM 780 confocal microscope. Images were processed using Zen Black (Zeiss) and then ImageJ. Cell counts were performed on images obtained from confocal imaging using the Cell Counter plugin for ImageJ (imagej.net/Cell_Counter). Synovial regions were categorized as ‘lymphoid aggregates’ when the B cells and T cells formed distinct clusters, and ‘diffusely infiltrated’ when B cells were loosely distributed within the synovium.

Statistical analyses

Statistical tests were performed as indicated in figure legends using two-sided tests. Dunn’s test was used for multiple comparisons in non-parametric tests and Bonferroni test for ANOVA. P-values <0.05 were considered significant.

36 Finck, R. *et al.* Normalization of mass cytometry data with bead standards. *Cytometry. Part A : the journal of the International Society for Analytical Cytology* **83**, 483-494, doi:10.1002/cyto.a.22271 (2013).

37 Finak, G., Perez, J. M., Weng, A. & Gottardo, R. Optimizing transformations for automated, high throughput analysis of flow cytometry data. *BMC bioinformatics* **11**, 546, doi:10.1186/1471-2105-11-546 (2010).

38 Bray, N. P., H.; Melsted, P.; Pachter, L. . Near-optimal RNA-Seq quantification. *arXiv* **1505**, 02710v02712 (2015).

FIGURE LEGENDS:

Figure 1: Expanded PD-1^{hi} $CXCR5^{-}$ $CD4^{+}$ T cells in joints and blood of seropositive RA patients.

a) viSNE plots of RA synovial tissue total $CD4^{+}$ T cells analyzed by mass cytometry. Color indicates cell expression level of labeled marker. Dotted circle indicates PD-1^{hi} cells. Arrow indicates $CXCR5^{+}$ cells. b) PD-1^{hi} T cell frequency in RA synovial tissue by mass cytometry (n=6). c) viSNE plots of paired RA synovial fluid and blood memory $CD4^{+}$ T cells. d) Flow cytometric detection of synovial fluid PD-1^{hi} $CD4^{+}$ T cells. e) PD-1^{hi} $CD4^{+}$ T cell frequency in synovial fluid from seropositive RA (n=9) and seronegative inflammatory arthritides (n=19). f) Flow cytometry for PD-1 and CXCR5 on memory $CD4^{+}$ T cells. g) Frequency of PD-1^{hi} cells in seropositive RA synovial fluid (n=9) and tissue (n=10). h) Frequency of PD-1^{hi} $CXCR5^{-}$ cells in seropositive RA (n=42), seronegative RA (n=16), spondyloarthropathies (SpA, n=11), and control (n=35) patient blood. i) PD-1^{hi} frequency in seropositive RA patients with low (n=14) or moderate-high (n=28) disease activity. j) PD-1^{hi} $CD4^{+}$ T cell and plasmablast frequencies before and after RA treatment escalation (n=18). Mean ± SD in b,e,g, median ± interquartile range in h,i shown. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ by Mann-Whitney (e,g), Kruskal-Wallis (h,i), Wilcoxon test (j).

Figure 2: Synovial PD-1^{hi} $CXCR5^{-}$ $CD4^{+}$ T cells express factors associated with B cell help.

a) RT-PCR for cytokines (n=7 donors) and intracellular regulators (n=5 or 6 donors) in T cell populations from seropositive RA synovial fluid. Median ± interquartile range. b) Flow cytometric quantification of IL-21, IL-2, and CXCL13 production by stimulated synovial $CD4^+$ T cell (n=3 experiments using different donors). c) Flow cytometric quantification of transcription factor expression in $CD4^+$ T cells from RA synovial fluid (blue, n=3 donors) or synovial tissue (green, n=3 donors). For b,c, mean ± SD shown. * p<0.05, ** p<0.01, *** p<0.001, **** p<0.0001 by Friedman's test compared to PD-1^- MHC II^- cells (a) or one-way ANOVA comparing PD-1^- $CXCR5^-$, PD-1^{hi} $CXCR5^-$, and PD-1^- $CXCR5^+$ (c).

Figure 3: High dimensional analyses of PD-1^{hi} $CXCR5^-$ and PD-1^{hi} $CXCR5^+$ cells identify shared and distinct features.

a) viSNE plots of blood memory $CD4^+$ T cells from a representative RA patient. Circle indicates PD-1^{hi} cells. b) Transformed median expression difference of significantly altered proteins between PD-1^{hi} populations and PD-1^- $CXCR5^-$ memory $CD4^+$ T cells (n=14 RA patients). c) Median expression of indicated proteins (n=7 RA patients (black) and 7 controls (grey)). d) PCA of RNA-seq transcriptomes (n=4 donors). e,f) Heatmap of expression of Tfh-associated genes (e) or chemokine receptors (f) by RNA-seq. g) Flow cytometric quantification of chemokine receptor expression on blood memory $CD4^+$ T cells. h) CCR2 expression on PD-1^{hi} $CXCR5^-$ $CD4^+$ T cells in RA synovial samples (tissue n=10, fluid n=5). For c,g,h, mean ± SD shown. * p<0.01, ** p<0.001, *** p<0.0001 Wilcoxon test.

Figure 4: PD-1^{hi} CXCR5^{-} cells promote plasma cell differentiation via IL-21 and SLAMF5 interactions.

a) Flow cytometric detection of plasma cells. b) Plasma cell frequency in co-cultures of memory B cells with indicated T cell populations from indicated sources.

Pooled data from 2 experiments (synovial tissue, n=3 replicates per experiment), 3 experiments (synovial fluid), or 6 experiments (blood) using different donors. c) Co-cultures using blood T cell subpopulations as in (b). d) Total IgG in supernatants of co-cultures as in (b). e,f) Co-cultures as in (b) with IL-21R-Ig fusion protein (e) or anti-SLAMF5/SLAMF6 antibody (f). For c-f) 1 of 3 experiments with different donors (n=3 replicates) shown. g,h) Immunofluorescence microscopy of RA synovium showing PD-1^{hi} CXCR5^{-} cells (white arrow) and PD-1^{hi} CXCR5^{+} cell (gray arrow). Scale bar = 50 microns. i,j) Quantification of PD-1^{hi} cells (i) and PD-1^{hi} cells adjacent to B cells (j) in RA synovium (n=5-8 HPF from 4 samples). Means ± SD shown. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ Mann-Whitney (b, synovial tissue), Kruskal-Wallis compared to PD-1^{-} CXCR5^{-} (b,c,e,f), or Wilcoxon (g).

Extended Data Figure 1: Detection of PD-1^{hi} CD4^{+} T cells in RA synovial tissue and fluid by mass and flow cytometry.

a) viSNE plots of mass cytometry data on CD4^{+} T cells as in Fig. 1a from two additional seropositive RA synovial tissue samples. b) Gating strategy to identify synovial tissue PD-1^{hi} CD4^{+} T cell populations by mass cytometry. c) Gating strategy to identify synovial fluid PD-1^{hi} memory CD4^{+} T cells by flow cytometry. d) Examples of gating used to sort memory CD4^{+} T cell populations from patient samples. e) Detection of CXCR5 mRNA by RT-PCR in sorted memory CD4^{+} T cell populations from synovial tissue (n=3 donors, 2 of which provided sufficient PD-1^{hi} CXCR5^{+} cells for analysis), synovial fluid (n=3 donors, 1 of which provided sufficient PD-1^{hi} CXCR5^{+} cells for analysis), and blood (n=2 donors). Purple boxes indicate PD-1^{-} and PD-1^{hi} CXCR5^{+} cells sorted from human tonsil as controls. Lines in (e) indicate mean for synovial or blood samples.

Extended Data Figure 2: PD-1^{hi} CXCR5^{-} CD4^{+} T cells are expanded in circulation of patients with active, seropositive RA and decrease with response to therapy.

a) Mean expression of MHC II and ICOS in memory CD4^{+} T cell populations defined by PD-1 and CXCR5 expression from synovial tissue (n=10), synovial fluid (n=9), and blood (n=42) from seropositive RA patients. Mean ± SD shown. b) Flow cytometric detection of PD-1 and CXCR5 expression on blood memory CD4^{+} T cells. c) Frequency of PD-1^{hi} CXCR5^{+} cells within circulating memory CD4^{+} T cells in patients with seropositive RA (RA Ab^{+}, n=42), seronegative RA (RA Ab^{-}, n=16), spondyloarthropathies (SpA, n=11), and non-inflammatory control patients (control, n=35) as in Fig. 1h. d,e) Frequency of PD-1^{hi} subpopulations that co-express MHC II or ICOS (d) or with intermediate PD-1 expression (e) in patients as in (c). f) Correlation between age or disease duration and circulating PD-1^{hi} $CXCR5^-$ $CD4^+$ T cells in all seropositive patients for which data was available (n=38). g) PD-1^{hi} $CXCR5^-$ T cell frequencies in seropositive RA patients segregated based on sex or medication usage (n=38). h) Correlation between serum anti-CCP antibody titer and circulating PD-1^{hi} $CXCR5^-$ $CD4^+$ T cell in all RA patients (n=53, black line, p=0.0049) or in only anti-CCP $antibody^+$ patients (n=29, green line, p=0.48). i) Correlation between fold change in CDAI and fold change in PD-1^{hi} $CXCR5^-$ T cell frequency patients 3 months after addition of a new RA medication (n=23; methotrexate=11, anti-TNF=4, abatacept=4, tocilizumab=2, tofacitinib=2). j) Frequency of PD-1^{hi} T cell subpopulations before and after RA treatment escalation in 18 patients with reduced disease activity after therapy. Median ± interquartile range in c,d,e, and mean ± SD in a,g is shown. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$ by Kruskal-Wallis (c,e), Mann-Whitney (d,g), Wilcoxon test (j). In f,h,i p-values calculated by Spearman correlation.

Extended Data Figure 3: Blood PD-1^{hi} $CXCR5^-$ $CD4^+$ T cells express factors associated with B cell help.

a) mRNA expression levels of cytokines/chemokines (n=10 donors, 6 RA patients (black), 4 controls (grey)) or transcription factors/signaling molecules (n=4 or 5 donors) detected by RT-PCR in sorted circulating memory $CD4^+$ T cell populations, normalized to RPL13A. Median ± interquartile range shown. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ Friedman's test, compared to PD-1^- $MHCII^-$ group. b) Cytokine and transcription factor mRNA expression in blood PD-1^{hi} $CD4^+$ T cell populations divided according to CXCR5 expression, relative to PD-1^{-} memory CD4^{+} T cells (n=6 donors). Mean ± SD shown. c) Flow cytometric quantification of of Bcl6 and Blimp-1 in PD-1^{hi} memory CD4^{+} T cell subpopulations sorted according to chemokine receptor expression, then stimulated in vitro for 2 days with anti-CD3/CD28 beads. Representative data from 1 of 3 experments using cells from different donors.

Extended Data Figure 4: Identification and characterization of circulating PD-1^{hi} CXCR5^{-} and PD-1^{hi} CXCR5^{+} in mass cytometry and RNA-seq analyses.

a) Gating of blood PD-1^{hi} memory CD4^{+} T cells in mass cytometry analyses. b) Flow cytometric detection of FoxP3 and PD-1 in blood memory CD4^{+} T cells from RA patients (black circles, n=5) and controls (grey circles, n=3). * $p<0.05$, **$p<0.001$ Kruskal-Wallis test compared to PD-1^{-} cells. c) Flow cytometric detection of inhibitory receptors on blood memory CXCR5^{-} CD4^{+} T cells. Data from 1 of 3 RA patients with similar results. d) Sort strategy for PD-1^{hi} CXCR5^{-} and PD-1^{hi} CXCR5^{+} cell populations for RNA-seq. e) Hierarchical clustering of T cell subsets sorted as in (d), with clustering based on expression of Tfh-associated genes measured in RNAseq transcriptomes.

Extended Data Figure 5: Limited interconversion of PD-1^{hi} CCR2^{+} and PD-1^{hi} CXCR5^{+} T cells *in vitro*.

a) Flow cytometry of CXCR5 and CCR2 on gated PD-1^{hi} $CD4^+$ cells from blood. b) Expression of CXCR5 and CCR2 on indicated sorted PD-1^{hi} T cell populations 7 days after *in vitro* stimulation with anti-CD3/CD28 beads. c,d) Percentage of cells from each sorted PD-1^{hi} population that expressed CXCR5 or CCR2 on day 2 (c) or day 7 (d) after *in vitro* stimulation. Naive $CD4^+$ T cells are shown as control. Mean ± SD shown (n=3 donors from 3 separate experiments).

Extended Data Figure 6: SLAMF5 is required for B cell-helper function of PD-1^{hi} $CXCR5^-$ $CD4^+$ T cells.

a) Flow cytometric quantification of SLAM, SLAMF5, and SLAMF6 expression on memory $CD4^+$ T cells (n=10 donors, 5 RA patients, 5 controls). b) Quantification of frequency of memory B cells with plasma cell markers after co-culture with PD-1^{hi} $CXCR5^+$ $CD4^+$ T cells with addition of blocking antibodies against SLAMF5 and/or SLAMF6. c) IgG quantification by ELISA in co-cultures of memory B cells with PD-1^{hi} $CXCR5^-$ or PD-1^{hi} $CXCR5^+$ $CD4^+$ T cells with addition of blocking antibodies against SLAMF5 and/or SLAMF6. For b,c) 1 of 3 experiments with similar results (n=3 replicates shown). Mean ± SD shown. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ Kruskal-Wallis compared to PD-1^- $CXCR5^-$ (a) or isotype control (b,c). d) Immunofluorescence microscopy of CD20 (green), CXCR5 (red), and PD-1 (blue), in seropositive RA synovial tissue. Arrows point to PD-1^{hi} $CXCR5^-$ cells adjacent to B cells. Scale bar = 50 microns.